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13. ABSTRACT (Maximum 200 words)

The regulation of synaptic reactivity by protein kinase C and its substrates has been studied using the long-term potentiation paradigm (LTP). We have studied the effects of protein kinase C activators and inhibitors on behavior: imprinting in the chick and radial arm maze performance. The main conclusion to be drawn is that PKC is necessary but not sufficient for the enhanced durability of memory. In combinatio with a neural signal, however, PKC demonstrates a profound synergism. Synergism is also observed in the analysis ofmetal ion regulation of PKC activity. Calcium and zinc interact in their effect on the enzyme in a bidirectional manner. Significant accomplishments made during the period were: determining the effect of inhibitors on behavior and the species generality of PKC-F1 module in memory formation.

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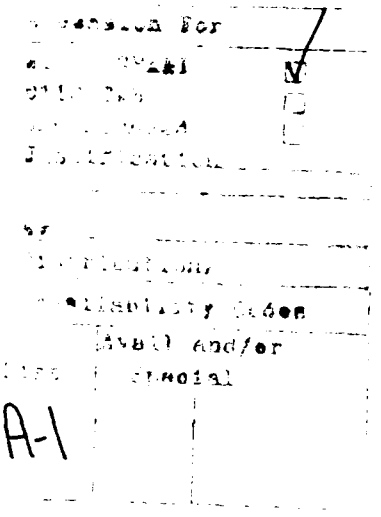

Air Force Office of Scientific Research

Annual Technical Report

1 March 1990 - 28 February 1991

Phosphoprotein Regulation of Synaptic Reactivity

Annual Technical Report for AFOSR 90-0240

Submitted to:
Directorate of Life Sciences
Air Force Office of Scientific Research

Submitted by:
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Annual Technical/Scientific Report

1. Project Period The project period includes March 1, 1990 to February 28, 1991. The present report is being filed in May, 1991.

2. Summary

The regulation of synaptic reactivity by protein kinase C (PKC) and its substrate proteins has been studied using a model of learning and memory, the long-term potentiation paradigm (LTP), biochemical analysis of purified enzymes and cellular analysis of ionic currents. A major focus of this work has been on the behavioral importance of this mechanism for developmentally-relevant behaviors. Two major projects are in the final stages of completion. The first project involves imprinting in 1 day old chicks. The second involves spatial learning in adult rats. In both cases we have demonstrated that protein kinase C (PKC) plays a signficant role in the process of learning. In the former case, a PKC substrate identified in 2-D gels as the MARCKS (myristoylated alanine rich C kinase substrate) is increase in its phosphorylation in relation to filial behavior. In the latter case a PKC inhibitor has a selective effect on memory for recently learned rules and procedures. This result may require a new categorization of memory processes.

3. Statement of Work

The research objectives during this period were to study:

A - The effect of imprinting on protein phosphorylation in specific brain regions of the chick

B - The effect of PKC inhibitors injected into hippocampus on spatial memory processes.

4. Status of research

Significant accomplishments made during this period were:

Behavioral regulation by protein kinase C

a. Imprinting in the Chick

Protein kinase C (PKC) substrates in chick brain were first identified. In collaboration with Gabriel Horn and Brian McCabe at Cambridge University we have identified 3 major PKC substrates in the chick brain. All 3 are acidic and correspond closely to the major substrates described by Nelson et al. (1989) in growth cones. These are the MARCKS protein, a 68kD molecule, protein F1, 50 kD in the chick and a 40kD protein which we have observed in neonatal and embryonic rat brain but which is not apparent in the adult. These proteins have the same microheterogeneity as seen on 2D gels and show intense phosphorylation when purified PKC is added to a sample of heat inactivated chick brain. Under

these conditions only the three proteins are seen to be phosphorylated.

In the study just completed two groups of chicks were run. One was exposed to a signficant stimulus (a rotating red light) whiler the other group was kept in the dark. The trained group was also studied with respect to the activity levels it showed in the presence of the imprinting stimulus and with respect to its preference for that stimulus vis a vis another stimulus to which it was not imprinted.

After the completion of the study and recording of the behavioral measurements, the chicks were killed by rapid decapitation and the brain dissected so that regions previously implicated in the process of imprinting were dissected from regions previously excluded from involvement. Specifically, the visual Wulst does not appear to be critical for the process while the intermediate region of the hyperstiratum ventrale (IMHV) has been implicated (Horn, 1988). Interestingly, only the left IMHV has been given an important functional role, so in the present study we compared the role of the two.

We have found that there is a significant increase in the phosphorylation of the MARCKS protein in the left IMHV in animals that have been imprinted vs. animals that have been reared in the dark. Interestingly, there was no effect of training on protein F1 phosphorylation. Nor were alterations detected in any other brain region studied or in any other protein studied. Thus, a selective unilateral alteration in a PKC substrate, the MARCKS protein, has been observed. It is particularly interesting that like the mammal the alteration detected is in a PKC substrate. But unlike the mammal, the bird shows a selective change in one substrate (MNARCKS) but not another (F1/GAP43).

b. Role of PKC in spatial memory

Since we have shown that PKC inhibitors block LTP and since LTP is considered a model of learning, it is reasonable to ask whether PKC inhbitors will have an effect on learning itself. For this purpose we have made use of the 8-arm radial maze which we and others have shown is highly sensitive to manipulation of the hippocampus.

Animals are first trained to seek a piece of food located in one of the eight arms. After finding it a delay of 1 min is interposed before the animal must go back to the same location to find the food again. Note that rats are foragers and thus are "prepared" to go to those locations that they have not visited previously. This type of behavioral pattern is opposed to the training which we impose. Thus, the animal has to overcome natural tendencies in order to master the new task. In this regard one is reminded that there are a variety of skills in which the normal response must be suppressed, e.g., in skiing the natural tendency when looking down a steep slope is lean up the hill; this will produce a most undesirable effect. In the radial arm maze the animal must overcome his tendency to

go to the other arms and learn to go the arm where food was found on the first trial. The animal is learning two things: one, where the food its and two, the strategy itself which is "go back to the arm where food was found."

After these skills are mastered, the rat then is anesthetized and bilateral indwelling cannulae are placed in the hippocampus. Recovery from surgery is followed by re-training and then injection of vehicle 30 min before trial 1. This is shown to have no effect. Then we inject a 1 ul volume of a 1, 5, or 10 mM solution containing polymyxin B, an inhibitor of PKC that works on the regulatory subunit. We observe the effects of the inhibitor for several days until normal performance returns. The placement of electrodes is then determined in post-mortem examination of formaldehyde fixed tissue.

We have found that PMXB has a profound effect on the ability of the animal to remember the location of the food on the second trial. What makes this important is that there is no effect on the ability of the rat to search for the food, the foraging behavior. Moreover, the effect is not permanent as the animal appears to be normal the next day.

As noted earlier, the animal learns two things, where the food is and the new strategy of going back to that location, overcoming its natural tendency to explore the alternative arms. It is reasonable to ask whether the impairment is in one or the other strategy. One can also view the first type of memory as closely related to the initial enhancement induced by an LTP-like process. The second type of memory is more closely related to a long-term storage process. If the first is the case, then we would imagine that it is acting postsynaptically, since we have recently shown that PKC post-synaptically is important in the earliest stages (0-15 min) of the development of LTP (Huang, Colley and Routtenberg, submitted).

5.Articles published, accepted for publication and submitted.

Published

1. Routtenberg, A. Role of protein kinase C and protein F1 in presynaptic terminal growth leading to information storage. In: H. Rahmann (Ed.) "Fundamentals of memory formation, "Progress in Zoology, 1990 Vol. 37, 283-295.

2. Sheu, F.-S., Kasamatsu, T., and Routtenberg, A. Elevated protein kinase C activity and substrate (F1/GAP-43) phosphorylation in kitten visual cortex parallels critical period. Brain Research, 1990, 524, 144-148.

3. Trommer, B. and Routtenberg, A. Long-term potentiation

in intact infant rat hippocampus. Developmental Brain Research, 1990, 53, 288-290.

4. Colley, P.A., Sheu, F.-S. and Routtenberg, A. Inhibition of protein kinase C blocks two components of LTP persistence leaving initial potentiation intact. J. Neurosci., 1990, 10, 3353-3360.

5. Routtenberg, A. Protein kinase C activation leading to protein F1 phosphorylation may regulate synaptic plasticity by presynaptic terminal growth. Reprinted in Neurobiology of Learning and Memory, World Scientific Publishing Co., F. Shaw, J. McGaugh, and S. Rose (Eds.), 1991, pp. 382-396.

6. Routtenberg, A. Long-term synaptic modification by protein kinase C: Regulation by activators, inhibitors, and NMDA receptors. Advances in Biochemical Pharmacology, G. Biggio, (Ed.), 1990, pp. 1-13.

7. Routtenberg, A. Trans-synaptophobia. Excitatory Amino Acids and Neuronal Plasticity, Y. Ben Ari (Ed.), 1990, Plenum Press, Volume 268, pp. 401-403.

8. Routtenberg, A. Action at a distance: The extracellular spread of chemicals in the nervous system. Volume Transmission in the Brain: New Aspect on Electrical and Chemical Communication, K. Fuxe (Ed.), 1991, Raven Press, 295-298.

9. Sheu, F.-S., Marais R.M., Parker, P.J., Bazan, N.G. and Routtenberg, A. Neuron-specific protein F1/GAP-43 shows substrate specificity for the beta subtype of protein kinase C. Biochem. & Biophys. Res. Comm., 1990, 171, 1236-1243.

In press

1. Florez, J.C., Nelson, R.B. and Routtenberg, A. Contrasting patterns of protein phosphorylation in human normal and Alzheimer brain: Focus on protein kinase C and protein F1/GAP-43. Experimental Neurology, 1991, in press.

2. Routtenberg, A. A tale of two contigent protein kinase C activators: Both neutral and acidic lipids regulate synaptic plasticity and information storage. Protein kinases and their substrates in brain, W. Gispen and A. Routtenberg (Eds.), 1991, in press.

3. Meberg, P.J. and Routtenberg. F1/GAP-43 expression: Selective distribution in developing and adult rat

brain. Neuroscience, 1991, in press.

4. Routtenberg, A. Transynaptophobia revisited. Long-Term Potentiation: A Debate of Current Issues, M. Baudry and J.L. Davis (Eds.), MIT Press, 1991, in press.

Submitted

1. Colley, P.A. and Routtenberg, A. A synaptic dialogue model of LTP: Role of pre- and postsynaptic protein kinase C. Submitted.

2. Huang, Y.Y., Colley, P.A. and Routtenberg, A. Postsynaptic then presynaptic protein kinase C activity is necessary for long-term potentiation. Submitted.

6. Personnel

Name	Title	Dates of Service	% Effort
A. Routtenberg	Professor/PI	9/83-present	25%
P. Colley	Post Doc	7/89-5/91	50%
F. Sheu**	Grad. Res. Asst.	9/84-6/1/91	50%
F. Cutting*	Grad. Res. Asst.	9/88-4/91	25%
Y. Huang	Visiting Scientist	4/89-11/90	100%
P. Meberg	Grad. Res. Asst.	9/87-present	50%
W. Kinney	Sr. Technician	4/91-present	50%
M. Desai	Res. Tech.	9/89-present	25%
Y. Chen	Res. Assoc.	7/91-present	50%

* - M.S. awarded 4/91
**- Ph.D awarded 6/91

7. Coupling Activities

1. Routtenberg, A. Invited speaker, Friedrich Miescher-Institut. "Protein kinase C and synaptic plasticity: New pathway for regulation." Basel, Switzerland, September 12, 1989. (Dr. A. Matus)

2. Routtenberg, A. Invited speaker. Wenner-Gren Symposium on Volume Transmission in the Brain: The Extracellular Fluid as a Pathway for Electrical and Chemical Communication. Stockholm, Sweden, September 27-30, 1989.

3. Routtenberg, A. Grass Traveling Scientist Lecturer in Neuroscience. "Molecular Mechanisms of Brain Information Storage." University of Illinois, Beckman Institute, November 14, 1989. (Dr. M. Gabriel)

4. Routtenberg, A. Invited speaker. Society of Toxicology symposium on "Cellular and Molecular

Mechanisms of Learning and Memory: Interactions and Neurotoxic Chemicals." Miami Beach, Florida, February 12, 1990. (Dr. H. Tilson)

5. Routtenberg, A. Invited speaker. Wayne State University, Detroit, Michigan, February 14, 1990. (Dr. J. Rafols)

6. Routtenberg, A. Invited speaker. Indiana University Bloomington, Indiana, February 16, 1990. (Dr. J. Farley)

7. Routtenberg, A. Invited speaker. New York University, New York, New York, April 9, 1990. (Dr. E. Azmitia)

8. Routtenberg, A. Invited speaker. Second Annual Midwestern Hippocampal Meeting, Northwestern University, June 30, 1990. (Dr. Nestor Schmajuk)

9. Routtenberg, A. Co-organizer and speaker. Third International Phosphoprotein Meeting. Utrecht, The Netherlands, August 23-26, 1990

10. Routtenberg, A. Invited speaker. Symposium on "LTP: A Debate of Current Issues". Gif s/Yvette, France, October 3-5, 1990. (Dr. Michel Baudry)

11. Routtenberg, A. "Human Behavior and Brain Function", McGill University, Department of Psychology, Montreal, Canada, November 22, 1990. (Dr. Michael Petrides)

12. Routtenberg, A. Invited speaker. Symposium on "Alzheimer's Disease: Status of Clinical and Basic Research." Mayo Clinic, Jacksonville, Florida, December 1 & 2, 1990. (Dr. Elliot Richelson)

13. Routtenberg, A. Invited speaker. Protein kinase C/Protein F1/GAP43: A molecular module of memory? Southern Illinois University School of Medicine, Springfield, Illinois, March 12, 1991. (Dr. Gregory Brewer)

14. Routtenberg, A. Invited speaker. Meeting on "Phospholipids and Signal Transmission", Wiesbaden, Germany, May 29-June 2, 1991. (Raphael Massarelli)

15. Routtenberg, A. Invited speaker. 13th ISN Meeting, Sydney, Australia, July 15-19, 1991. (Dr. R. Rodnight)

16. Routtenberg, A. Invited speaker. Department of Cell, Molecular, and Structural Biology, Northwestern University, "Membranes, Molecules, Memories, Modules and the Mind." Chicago, Illinois, April 18, 1991.

17. Routtenberg, A. Invited speaker. American Association of Anatomists 1991 annual meeting, "Cellular Mechanisms of Behavioral Plasticity." Chicago, Illinois, April 20, 1991 (Dr. Jonathon Jones).

18. Farley, J. and Routtenberg, A. LTP reduces K+ Channel activity in hippocampal synaptosomes. Society for Neuroscience, New Orleans, Louisiana, November 10-15, 1991, in press.

19. Meberg, P.J., McCabe, B.J., Horn, G., Rosenfeld, J.P. and Routtenberg, A. Differential mRNA distribution in chick brain of two protein kinase C (PKC) substrates, F1/GAP43 and Marcks. Society for Neuroscience, New Orleans, Louisiana, November 10-15, 1991, in press.

20. McCabe, B.J., Sheu, F.-S., Horn, G. and Routtenberg, A. Memory alters protein kinase C substrate (Marcks) phosphorylation. Society for Neuroscience, New Orleans, Louisiana, November 10-15, 1991, in press.

21. Sheu, F.-S., Azmitia, E.C., Marshak, D.R., Parker, P.J. and Routtenberg, A. Glial-derived S-100 protein selectively inhibits the neuron-specific protein F1/GAP-43 phosphorylation by beta 1 recombinant protein kinase C: Implications for a glial-neuronal interaction. Society for Neuroscience, New Orleans, Louisiana, November 10-15, 1991, in press.

8. New Directions/Discoveries

In the last year we have found in an initial study that dietary CUFAs can enhance learning and memory related performance. The possible application to performance enhancement in humans and the continued exploration of the best conditions for achieving enhancement in animals suggests a promising direction for future research. The abstract of this initial study follows.

Abstract (From Farley and Routtenberg, to be submitted)

Because potassium ion channels in presynaptic terminals are involved in the regulation of neurotransmitter release at many synapses, a persistent reduction in their activity induced by tetanizing stimulation might be expected to contribute to LTP. We have examined this possibility by incorporating hippocampal synaptosomal vesicle membranes, from animals in which LTP was induced into planar lipid bilayers. We observed a near-elimination of K+ channel activity in these membranes, as compared to that of sham and low-frequency stimulation controls. The possibility that this effect was mediated by protein kinase C was considered.